ABORTIVE INITIATION AS A BOTTLENECK FOR TRANSCRIPTION IN THE EARLY *DROSOPHILA* EMBRYO

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Abstract

The standard model of transcription — the totally asymmetric simple exclusion process (TASEP) — predicts the existence of a gene-independent limit on the maximal rate of transcription as a result of polymerase "traffic jams" in the bulk of the gene at sufficiently high polymerase concentrations. Recent experiments in living *Drosophila* embryos provide quantitative access to the transcriptional dynamics of genes in the high polymerase concentration limit. Our analysis suggests uniformity of the maximal rate of transcription in the genes *hunchback*, *snail*, and *knirps*, and their modified constructs. Intriguingly, the observed maximal rate of transcription is only about 40 % of the one predicted by the TASEP model. We propose that the appearance of a gene-independent maximum rate of transcription indeed hints at fundamental physical constraints on the process due to traffic jams, but the observed reduction in the maximal rate reflects jamming in the promoter region, where the polymerase elongation rate is lower due to cycles of abortive initiation. This implies that the transcription bottleneck is not in the bulk of the gene, but rather in its promoter region, and we suggest experiments to test this hypothesis. This provides an alternative transcription rate reduction mechanism compared to earlier promoter dynamics studies.

Photo credit: Nina

Motivation

- How fast can developing cells produce new material in early embryonic stages?
- Are there fundamental physical limits?

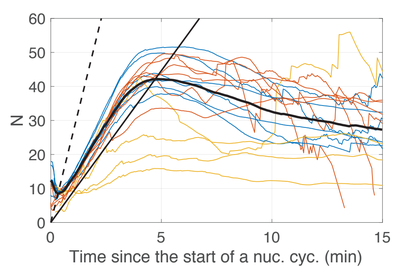
Photo credit: André Karwath

Data

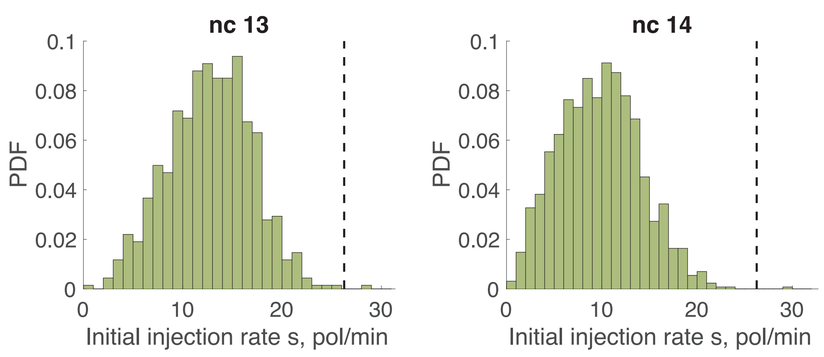
Number of analyzed fluorescence traces for different genes and constructs:

	hunchback	*snail*	*knirps*
bac	1965 (11)	547 (5)	326 (5)
no primary	979 (9)	607 (4)	84 (2)
no shadow	1279 (8)	159 (1)	60 (1)

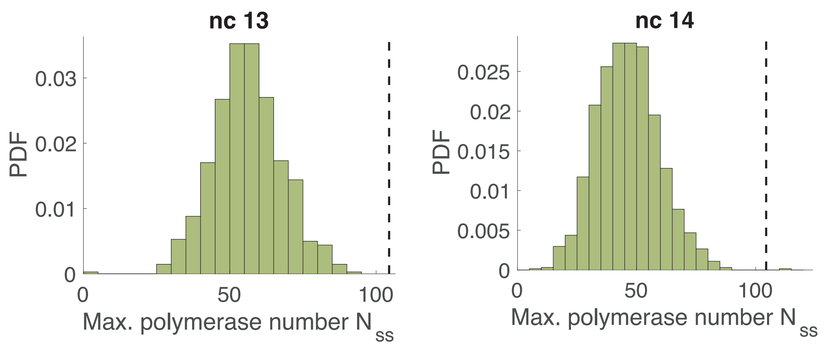
Mean evolution of the active polymerase number in a nuclear cycle for the *hunchback* (blue), *snail* (yellow) and *knirps* (red) genes:



Polymerase injection rates in nuclear cycles 13 and 14 for the *Hb* gene:



Steady-state active polymerase number in nuclear cycles 13 and 14 for the *Hb* gene:



The four distributions exhibit average values **~ 40 %** of the values predicted by the standard TASEP model in the maximal current regime.

Independent of anterior-posterior (AP) position

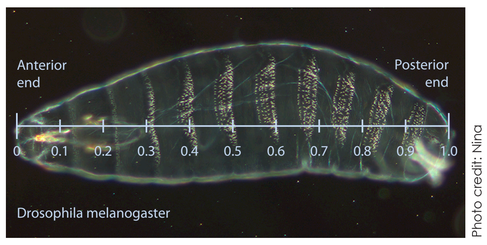
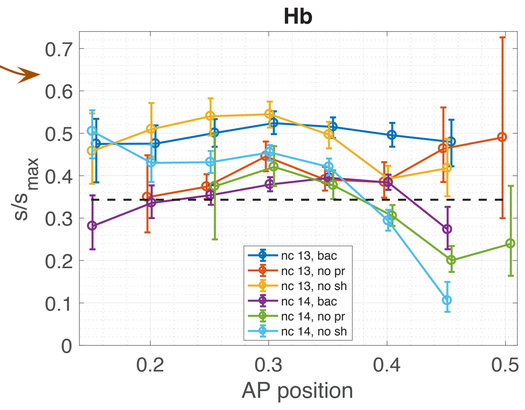


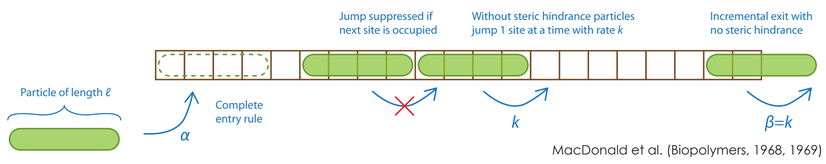
Photo credit: Nina

Pattern formation

Normalized polymerase recruitement rate on the *Hb* gene does not depend on AP, nc or enhancer construct!



Original ℓ-TASEP model



MacDonald et al. (Biopolymers, 1968, 1969)

Problems:

- the observed **injection rate** is ~ 40 % of the model prediction in the maximal current regime [2],

$$s_{\max} = \frac{k}{(1+\sqrt{\ell})^2}$$

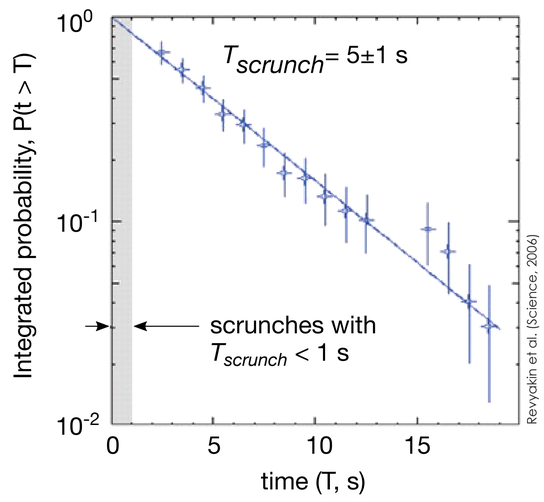
- the observed **steady-state polymerase (particle) density** is ~ 40 % of the model prediction in the maximal current regime [2].

$$\rho_{\max} = \frac{1}{\sqrt{\ell}(1+\sqrt{\ell})}$$

Hypothesis

The decrease in the injection rate is due to abortive injection.

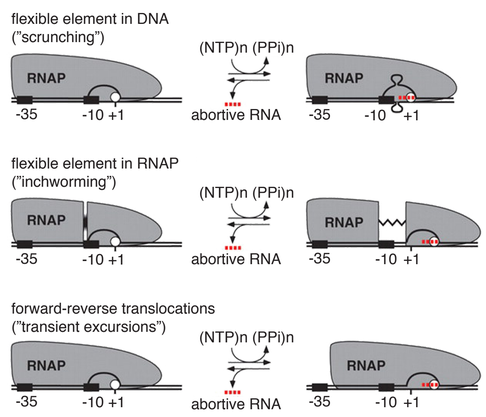
This effect can be modeled by making polymerases pause at the first site for a random amount of time.



Revyakin et al. (Science, 2006)

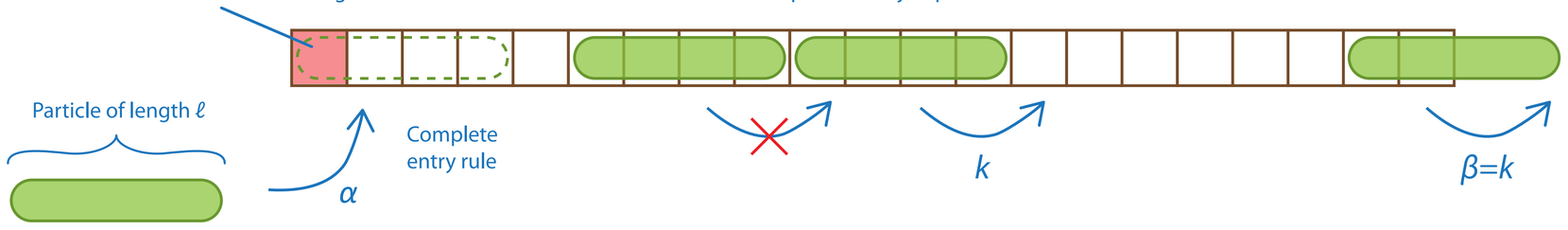
In vitro experiments show that the waiting time is exponentially distributed with the mean pause time $\tau = 5 \pm 1$ s.

The abortive initiation proceeds through a scrunching mechanism [3].



Revyakin et al. (Science, 2006)

The Abortive Model



New Predictions

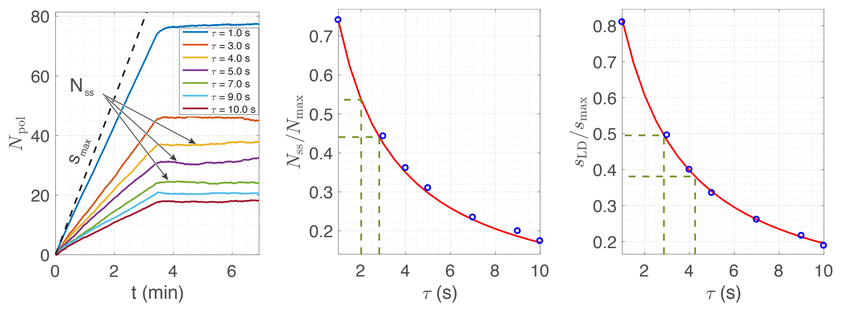
Reduced injection rate:

$$s/s_{\max} = \frac{(k\tau - 1)(1+\sqrt{\ell})^2}{k\tau(k\tau + \ell - 1)}$$

Reduced steady-state polymerase number:

$$N_{\rm ss}/N_{\max} \approx \frac{\sqrt{\ell}(1+\sqrt{\ell})}{k\tau + \ell - 1}$$

Our approximate formulas are validated by numerical simulations:



Comparison to Data

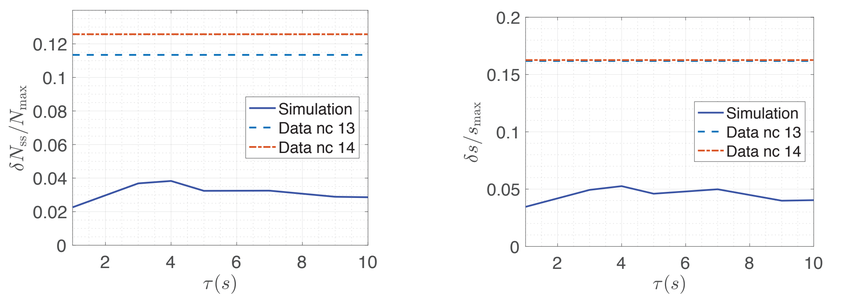
The predictions are in line with the experimental data:

Observable	nc	Abortive ℓ-TASEP	*hb* bac	*hb* no pr.	*hb* no sh.	*sn* bac	*sn* no pr.	*sn* no sh.	*kn* bac	*kn* no pr.	*kn* no sh.
$N_{\rm ss}/N_{\max}$	13	0.30	0.54 ± 0.13	0.4 ± 0.1	0.50 ± 0.11	0.46 ± 0.10	0.52 ± 0.11	0.49 ± 0.11	0.43 ± 0.11	–	–
	14	0.30	0.44 ± 0.13	0.4 ± 0.2	0.47 ± 0.12	0.49 ± 0.09	0.50 ± 0.11	0.49 ± 0.09	0.35 ± 0.13	0.16 ± 0.04	0.27 ± 0.08
$s/s_{\max}$	13	0.34	0.5 ± 0.2	0.4 ± 0.2	0.5 ± 0.2	0.4 ± 0.1	0.4 ± 0.2	0.4 ± 0.2	0.3 ± 0.2	–	–
	14	0.34	0.4 ± 0.2	0.3 ± 0.2	0.4 ± 0.2	0.3 ± 0.2	0.4 ± 0.2	0.4 ± 0.1	0.2 ± 0.1	0.11 ± 0.06	0.17 ± 0.10

One can estimate τ in the *in vivo* experiments:

Observable	nc	*hb* bac	*hb* no pr.	*hb* no sh.	*sn* bac	*sn* no pr.	*sn* no sh.	*kn* bac	*kn* no pr.	*kn* no sh.
$N_{\rm ss}/N_{\max}$	13	2.0 ± 0.9	3 ± 1	2.3 ± 0.9	2.6 ± 0.9	2.1 ± 0.9	2.3 ± 0.9	2.9 ± 1.2	–	–
	14	3 ± 1	3 ± 2	2.6 ± 1.2	2.3 ± 0.8	2.3 ± 0.9	2.4 ± 0.8	4 ± 2	10 ± 4	6 ± 2
$s/s_{\max}$	13	3 ± 2	4 ± 3	3 ± 2	5 ± 3	4 ± 2	3 ± 2	6 ± 4	6 ± 4	6 ± 4
	14	4 ± 3	6 ± 5	4 ± 3	5 ± 3	4 ± 3	5 ± 3	9 ± 7	20 ± 13	12 ± 8

Interestingly, the model predicts less noise than seen in experiment:



Conclusions

- Maximal transcription rate and polymerase density do not depend on specific gene, construct or nuclear cycle;
- The experimental measurements correspond to ~ 40 % of the predicted limits;
- Considering the abortive initiation effect is enough to account for this discrepancy.

References

1. MacDonald et al., *Biopolymers* 6, 1 (1968)
2. MacDonald and Gibbs, *Biopolymers* 7, 707 (1969)
3. Revyakin et al., *Science*, 314 (*5802*), 1139 (2006)